Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

NATURAL HEMOLYSINS IN NORMAL HORSE SERUM *

FRED BOERNER, JR.

From the Laboratory of the Pennsylvania State Livestock Sanitary Board, Philadelphia

During the study of the complement-fixation test for glanders in horses, our attention was attracted by the question of a natural hemolysin for the corpuscles of the sheep and the ox.

A search of the literature has failed to give us the desired information. Morgenroth and Sachs[1] state "it is worthy of note that in almost every horse serum which we examined for the purpose, we found a considerable amount of amboceptor for guinea-pig blood. The amboceptor was characterized by a particularly high degree of thermolability, being invariably destroyed by heating to 55 degrees C. A complement for the same is very often absent, and even when present it is only on the addition of considerable amounts of fresh guinea-pig serum that this amboceptor becomes manifest." Sachs,[2] in his table compiled from the literature, mentions as doubtful the presence of natural amboceptors for the erythrocytes of guinea-pigs and rabbits, and an absence of the same for the erythrocytes of other vertebrates.

Having sufficient material constantly at hand, we decided to determine this point to our own satisfaction. After the examination of a sufficient number of sera for the presence of antisheep and antiox hemolysins had been completed, it was thought of interest to continue the work with the erythrocytes of other vertebrates as follows: guinea-pig, hog, goat, cat, white mouse, man, rabbit, chicken, and dog.

TECHNIC

Serum.—The sera of normal horses used in these experiments were separated from blood collected from the jugular vein into sterile test-tubes, which during the clotting were kept in an upright position in the ice-chest. The clear serum was then removed and inactivated by heating to 60 C. for one-half hour to destroy the anti-complementary substances and to render inactive any native complement. At first the amounts of 0.4 c.c., 0.3 c.c., 0.2 c.c., 0.1 c.c., and 0.5 c.c. were employed. Later, after having observed the continued negative results obtained with sheep corpuscles, we abandoned the last four dilutions, using only

---

* Received for publication July 1, 1915.

[1] Ehrlich: Collected Studies in Immunity, 1906.

[2] Kolle and Wasserman's Handb. d. pathogen. Microorganismen, 1913, 2,^{2} p. 793.

one tube for each serum in the dose of 0.4 c.c., with the intention of retesting in higher dilutions should any show traces of hemolysis with this amount.

Complement.—Fresh guinea-pig serum was employed as a complement in the dose of 1 c.c. of a 5% normal salt solution. In each case the complement was tested for the presence of native amboceptors for the erythrocytes to be tested, and it was found that guinea-pig serum frequently possessed sufficient antidog hemolysin to produce traces of hemolysis when used in the dose of 1 c.c. of a 1 : 20 dilution. However, we have not classed this in the category of amboceptors for the reason that treatment with analogous cells failed to remove the hemolysin. Amboceptors for the various other cells tested were not sufficient to produce any hemolysis when used in this dilution.

Erythrocytes.—With the exception of that from sheep corpuscles, the corpuscle suspensions were always prepared from fresh blood and used within 24 hours from the time collected, after thorough washing. The sheep cells used varied in age from 1 to 8 days, the corpuscles of but one sheep being used. The blood of this animal had previously been tested for its keeping properties and was found to keep from 2 to 3 weeks without becoming unduly fragile if preserved in its own serum and washed only as needed. In all the tests a dose of 1 c.c. of a 25% suspension of washed corpuscles was used.

TABLE 1

EXAMINATION OF HORSE SERA FOR NATIVE ANTISHEEP HEMOLYSIN

Amount of Serum; c.c.	100% Hemolysis	75% Hemolysis	50% Hemolysis	25% Hemolysis	0 Hemolysis
0.4	3	0	1	0	196
0.3	0	1	1	2	196
0.2	0	0	0	2	198
0.1	0	0	0	0	200
0.05	0	0	0	0	200

Salt Solution.—Enough salt solution (0.85%) was used in each tube to make a total volume of approximately 3 c.c. Control: The maximal dose of serum was tested with the erythrocytes used to determine whether inactivation was complete. These sera were also tested with the anti sheep hemolytic system to make sure that they were not anti-complementary. The usual dose of complement was tested with the erythrocytes to make certain that all the native amboceptors were removed. The corpuscle suspension was controlled in normal salt solution for undue fragility. Readings: All readings were made at the end of 2 hours' incubation at 37 C. The control tubes, however, were placed in the ice-chest and examined on the following morning.

Antisheep Hemolysins.—The results of the examination of 200 horse sera for native antisheep hemolysin are shown in Table 1. Of the 200 sera tested, but 4 gave any trace of hemolysis. Of these, 3 gave complete, and one 50% hemolysis in dilutions of 0.4 c.c. The remaining 196 were completely negative. Converting the figures which represent the number of sera into percentages, we find but 1.5% capable of producing complete hemolysis with 0.4 c.c. of serum, and 98% entirely negative as regards any signs of hemolysis.

For fear that the animals the sera of which were positive in the test for amboceptors might possibly be suffering from some disease, a careful clinical examination was made, which showed them to be in perfect physical condition.

Antidog Hemolysin.—During the study of these cells we encountered the presence of natural hemolysin in guinea-pig serum used as complement. The failure to remove it by the usual methods employed, led us to a series of experiments with these cells which resulted in finding them subject to a very peculiar hemolysis. For example, guinea-pig serum, as well as horse serum, was found capable of producing hemolysis of these corpuscles without the activity of a complement. A more detailed report of these experiments will be published later.

Examination of other Bloods.—The cells of other vertebrates were tested with one dose of serum, namely, 0.4 c.c. All the essential data, including the various bloods employed, the number of sera tested, and the results obtained with this amount, have been summarized in Table 2.

TABLE 2

SUMMARY OF NATURAL HEMOLYSINS IN HORSE SERUM (0.4 C.C. AMOUNTS)

Blood	Number of Sera Tested	Percentage Showing 100% Hemolysis
Sheep	200	1.5
Ox	100	0
Guinea-pig	50	0
Hog	50	0
Goat	50	0
Cat	50	0
Mouse (white)	50	0
Rabbit	50	0
Chicken	50	0
Man	50	0

CONCLUSIONS

Of 200 sera examined for antisheep hemolysin, 1.5% were found capable of producing complete destruction of these cells in the amount of 0.4 c.c.

Horse serum was found to possess the property of dissolving the red cells of the dog without the aid of complement.

Native hemolysins for the cells of guinea-pig, hog, goat, cat, white mouse, rabbit, chicken, and man were not found.